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**Enhancement of the formulation of “almendrado” biscuit through
the incorporation of almond oil extraction by-products**

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É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTA DISSERTAÇÃO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO DO INTERESSADO, QUE A TAL SE COMPROMETE.

AUTORA:

ORIENTADOR:

COORIENTADORA:

“As nuvens mudam sempre de posição, mas são sempre nuvens no céu. Assim devemos ser todos os nossos dias, mutantes, porém leais com o que pensamos e sonhamos; lembra-te, tudo se esvanece no ar, menos os teus pensamentos.”

Paulo Beleki

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ABSTRACT

Meeting the needs of current generations without compromising the future of next, is one of the cornerstones of the sustainability concept. The fast growing food processing industry generates high volumes of by-products and their recovery can be extremely important to reduce the negative impact on the environment and to provide new economic trends.

Furthermore, there is an increasing concern about food properties, which is changing dietary patterns towards the consumption of healthier products. Nuts are a striking example of the so called functional foods. Among them, almonds are greatly used in food industry, being one of the most nutritious and versatile nuts, renowned for its health benefits and culinary uses. One of the current applications of almond is the extraction of its oil for high-specialty culinary purposes. The partially delipidified flour that remains after the extraction process has the potential to be applied in advantageous uses. Accordingly, this almond by-product was thoroughly studied and further used in the development of a new, healthier and enhanced formulation of a traditional and highly appreciated almond-base cookie: “almendrado”.

The proximate composition, fatty acids and tocopherols profiles were characterized and compared in different formulations: i) industrial (AI); ii) produced according to the traditional recipe (AT); iii) AT added with partially delipidified flour (AF); and iv) AF with a 30% sugar reduction (AFSR). In addition, the sensory quality and consumer acceptance were evaluated by a group of 74 tasters that answered a questionnaire based in hedonic scale questions.

Carbohydrates were the major nutrient in all “almendrados”, followed by fat or protein, water and ash. AI has the highest fat content and lower levels of protein, carbohydrates and water, when compared to AT, AF and AFSR. Among traditional-based formulations, AF and AFSR showed significantly higher contents in protein and mineral elements and the lowest energy values. Among individual compounds, AI showed the highest vitamin E values (7.2 ± 0.3 mg/100 g fw), whilst AF showed the lowest (3.1 ± 0.2 mg/100 g fw). α -Tocopherol was the most abundant vitamer in all formulations. Regarding fatty acids profiles AT, AF and AFSR showed high similarity, presenting $\approx 70\%$ of MUFA and $\approx 20\%$ of PUFA; oleic acid was clearly the most abundant FA, followed by linoleic acid and palmitic acids. AI presented a high percentage of SFA, mostly due to the contribution of palmitic acid and stearic acid.

The sensory analysis gave highly positive results in all criteria: appearance, overall taste, sweetness, crunchiness, hardness, global quality and buying predisposition. The traditional recipe based formulations gave the best results, with particular relevance for AF, clearly indicating of the enhanced effect achieved with the incorporation of the PDAF by-product.

Keywords: almond; by-product; nutritional profile; vitamin E; fatty acids; sensory analysis.

RESUMO

A resposta às necessidades das gerações atuais sem comprometer as futuras é uma das premissas fundamentais do conceito de sustentabilidade. Neste contexto, as quantidades massivas de sub-produtos gerados na indústria alimentar têm de ser reutilizadas para reduzir o impacto ambiental e promover novas abordagens económicas nas indústrias.

A crescente preocupação com os efeitos fisiológicos dos alimentos está a alterar os padrões dietéticos, promovendo o consumo de alimentos mais saudáveis. Entre os chamados alimentos funcionais encontra-se a amêndoa; a sua utilização indústria alimentar é elevada, sendo muito versátil, nutritiva e reconhecida pelos seus diversos benefícios para a saúde. Uma das aplicações atuais da amêndoa é a extração de óleo para fins culinários de elevada especialidade. A farinha parcialmente deslipidificada (FPD) que remanesce após a extração poderá ser utilizada em aplicações vantajosas. Assim, este sub-produto da amêndoa foi caracterizado e posteriormente utilizado no desenvolvimento de uma formulação otimizada e mais saudável de um biscoito de amêndoa altamente apreciado: o almendrado.

A composição nutricional e os perfis de vitamina E e ácidos gordos foram comparados em diferentes formulações: i) almendrado industrial (AI); ii) almendrado produzido de acordo com a receita tradicional (AT); iii) AT com farinha de amêndoa parcialmente deslipidificada (AF); iv) AF com redução de 30% de açúcar (AFSR). A qualidade sensorial e a aceitabilidade foram avaliadas por um grupo de 74 provadores através de questões com escala hedónica.

Os hidratos de carbono foram o nutriente maioritário, seguindo-se a gordura ou proteína, a água e os minerais. O AI apresentou o maior conteúdo em gordura e níveis mais baixos de proteína, hidratos de carbono e água. Entre as formulações de base tradicional, os AF e AFSR apresentaram maiores teores de proteína e minerais, mas menores valores calóricos. O AI revelou o maior conteúdo em vitamina E (7.2 ± 0.3 mg/100 g fw), enquanto AF apresentou o mais baixo (3.1 ± 0.2 mg/100 g fw). O α -tocoferol foi o vitâmero mais abundante em todas as formulações. Em relação aos ácidos gordos, os AT, AF e AFSR apresentaram perfis similares, com $\approx 70\%$ de AGPI e $\approx 20\%$ de AGMI; o ácido oleico foi o mais abundante, seguido pelo ácido linoleico e o ácido palmítico. Por outro lado, o AI apresentou uma maior percentagem de ácidos gordos saturados (principalmente palmítico e esteárico).

Os resultados obtidos na análise sensorial foram altamente positivos em todos os critérios: aspeto, sabor, doçura, crocância, dureza, qualidade global e tendência de compra. Os melhores resultados registaram-se para os AT, AF e AFSR, com relevância para o AF, comprovando a melhoria conseguida com a incorporação do sub-produto FPD.

Palavras-chave: amêndoa; sub-produto; perfil nutricional; vitamina E; ácidos gordos; análise sensorial.

PUBLICATIONS AND COMMUNICATIONS

- [1] Silva, B.L.B.V.; Barreira, J.C.M.; Oliveira, M.B.P.P. 2016. Natural phytochemicals and probiotics as bioactive ingredients for functional foods: extraction, biochemistry and protected-delivery technologies. *Trends in Food Science and Technology*, 50, 144-158 (IF 5.150 - Q1). DOI: 10.1016/j.tifs.2015.12.007
- [2] Silva, B.L.B.V.; Barreira, J.C.M.; Oliveira, M.B.P.P. 2015. Extraction of bioactive compounds to develop effective functional foods. 8º IJUP, 8th Meeting of Young Researchers of University of Porto, 13-15 of May, University of Porto.
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ABBREVIATIONS

AF	(traditional “almendrados” with partially delipidified almond flour)
AFSR	(traditional “almendrados” with partially delipidified almond flour and with a 30% sugar reduction)
AI	(industrial “almendrados”)
ANOVA	(analysis of variance)
AOAC	(association of analytical communities)
AT	(traditional “almendrados”)
DAD	(diode array detector)
dm	(dry mass)
FA	(fatty acids)
FAME	(fatty acids methyl esters)
FD	(fluorescence detector)
FID	(flame ionization detector)
FPD	(farinha parcialmente deslipidificada)
fw	(fresh weight)
GC	(gas chromatography)
GC-FID	(gas chromatography-flame ionization detection)
HPLC	(high performance liquid chromatography)
IS	(internal standard)
LDL	(low density lipoprotein)
MUFA	(monounsaturated fatty acids)
PDAF	(partially delipidified almond flour)
PUFA	(monounsaturated fatty acids)
SFA	(saturated fatty acids)
TSG	(traditional specialty guaranteed)
UN	(United Nations)
USFDA	(United States Food and Drug Administration)
UV	(ultraviolet)

1. INTRODUCTION

The present context of climate change, ongoing loss of species and genetic diversity, soil degradation and increasing population, represent a urgent need of an integrated action to address food and nutrition security issues at a global level, meeting the needs of current generations without compromising the future of the next [1].

We all know that the resources provided by nature must be preserved with utmost care and following suitable management strategies, since they are not an inexhaustible source. This is one of the main reasons to the emergence of the sustainability concept [2]. This concept is continuously evolving, but it can be generally defined as a process that aims to reconcile the balance of ecosystems with man's ability to interact with the world and human needs for survival, preserving the environment, not compromising natural resources for future generations. This is obviously complex, since it comprises a set of independent variables that integrate social, energy, economic and environmental issues [3].

The idea of sustainability began to take shape in 1972, at the United Nations Conference on the Human Environment in Stockholm, where it was settled that "Man has the right to remain in an environment whose quality of life will allow him to live with dignity and well-being, and is responsible for the solemn duty to protect and improve the environment for current and future generations" [2]. Later, in "The United Nations Conference on Environment and Development" - ECO-92, held in Rio de Janeiro in 1992, the concept of sustainable development was consolidated, highlighting the importance of integrating two major themes: environment and development.

In the European Union, the "Three Pillars Sustainability Model" was developed based in the idea that sustainable development will only be achieved if environmental, economic and social development are symbiotically related. The three main objectives are: more value, less impact and better alternatives [4].

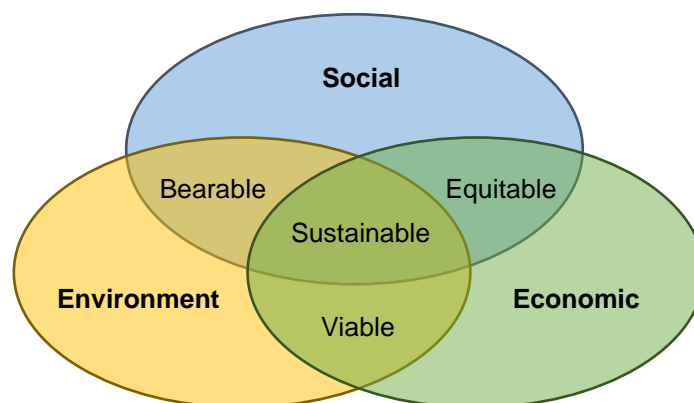


Figure 1. Three pillars sustainable model.

The sustainability principles were reiterated in the Rio +20 meeting, aiming to achieve a sustainable development, in order to ensure a future for the planet for present and future generations [5]. Economically, the use of sustainable processes should result in reducing costs and increasing revenue, through the knowledge of reusable by-products and their benefits to the health and safety of consumers. Recently, in 2015, the 17 Sustainable Development Goals were established at a UN summit in New York [2].

All the indicates premises were also adopted in food security dynamics, as evidenced by the emerging concept of “sustainable diets”, which has been proposed as a multidimensional framework to address the need for nutritious and adequate food in the context of the many challenges facing the world today: reducing poverty and hunger, improving environmental health, enhancing human well-being and health, sustainable livelihoods and cultural heritage [2]. Furthermore, the increasing worldwide population is demanding for an exponential growth in food products, a scenario aggravated by the continuous ecosystems degradation, which will drastically affect plant and animal resources [6].

Thereby, it is unclear how the current global food system will meet future needs of the growing demands of a population that might reach 9 billion individuals in 2050. In fact, one of the greatest challenges of our time is improving the current food system to provide adequate and nutritious food in an environmentally and socio-culturally sustainable manner.

Thus, it is imperative to take responsibility in developing sustainable products, implementing cleaner production systems and sustainable management systems, ultimately adopting the 3 R's (Reduce/Reuse/Recycle) [7].

Accordingly, the reutilization of food waste is vital, especially considering the huge amounts produced continuously (reaching up to 1.3 billion tons of edible materials wasted annually). The product reuse and the recovery of waste by-products can be extremely important to reduce the negative impact on the environment and also provide new economic trends for industries. Food by-products provide bioactive compounds with scientifically proven biological properties that can be integrated in different areas such as pharmaceutical, cosmetic and food [8].

In this context of circular economy, the present work intended to create an advantageous use for the food by-products generated in the industry of almond oil extraction. The partially delipidified almond flour (PDAF), which contain several bioactive compounds with potential health benefits, was used to improve the formulation of a highly appreciated specialty biscuit (“almendrado”). In addition to the enhanced sensorial properties and potential physiological effects, this approach resulted in the creation of a new economically advantageous resource for the oil extraction industry.

1.1 TOP TRENDS

During recent years, the changes in diets and lifestyles resulting from industrialization, urbanization, economic development and market globalization have increased rapidly, particularly in developing countries where rapid socioeconomic changes are occurring. Whereas improvements in the standards of living have been observed, this has often been accompanied by unhealthy dietary patterns and insufficient physical activity to maintain energy balance and a healthy weight. The net result has been an increased prevalence of diet-related chronic diseases in all socioeconomic groups [9, 10].

The global food consumption level is a major challenge to the achievement of sustainable production practices, due to its impact on the ecosystem, public and individual health, social cohesion and world economy. Population growth and increasing economic prosperity requires an excessive use of resources such as energy, food and water that compromises the sustainability of natural resources and can exacerbate social and political issues. Recent trends in global food production, distribution, and preparation call for increased research in food security, in order to ensure a safer global food supply [1].

Nowadays, nearly 800 billion people are affected by hunger in the world and have no access to clean water; on the other hand, 1.9 million adults are overweight (600 million obese), being part of a growing trend of diseases caused by unbalanced diets rich in sugar, animal protein and trans fats [1, 11]. The hunger problem is even more revolting if we consider that a third of the food produced globally is wasted [3, 11]. The current review of the sustainable development strategies in Europe and the action plans highlight key goals towards sustainable food consumption such as improving health and reducing obesity levels by increasing the consumption of organic food and drastically reducing food waste [2].

In addition, consumers are increasingly aware of diet related health problems, demanding natural ingredients which are expected to be safe and health-promoting. In response to this concern, there is a rapidly growing body of literature covering the role of plant species as potential sources of these ingredients [12]. Actually, there are some trends to reduce fats, increase protein content, substituting sugars by natural sweeteners, or using natural dyes, replacing “E” additives to avoid the perception of incorporating synthetic ingredients, generally intending to produce “green” and sustainable food with simple and clear labels, under campaigns focused in healthier lifestyles.

1.2 *PRUNUS DULCIS* (MILLER) D. A. WEBB (ALMOND)

In recent years, the growing concern about food properties led to an increase in the consumption of nuts, such as it is exemplified by almond. Almond belongs to the Rosaceae family that also includes plants such as plum, cherry, peach, apples, pears and raspberries. Due to its fruits of high commercial value, almond tree is the number one crop in tree nut production worldwide [13, 14].

Like many other Rosaceae fruit, almond trees are often self-sterile, requiring pollen from another cultivar before fruits can be produced [15]. They have brown or gray bark and either an erect or weeping growth habit depending on the variety of the tree. The trunk can reach 30 cm in diameter; the leaves are 7.5-13 cm long. The tree produces white to pale pink flowers that appear before the leaves in early spring; it produces also hairy green fruits which are oblong in shape. The fruit is a drupe, containing a single seed. The seed is protected by a hard brown shell. At maturity, the flesh of the fruit becomes leathery and splits to reveal the nut inside. Nuts generally measure 3.5 to 6 cm in length. Almond trees can reach heights between 4 and 10 m and have a commercial lifespan of between 30 and 40 years [16-18]. Almonds are grown as orchard crops, highly nutritious but owing to their high cost they are a luxury food.

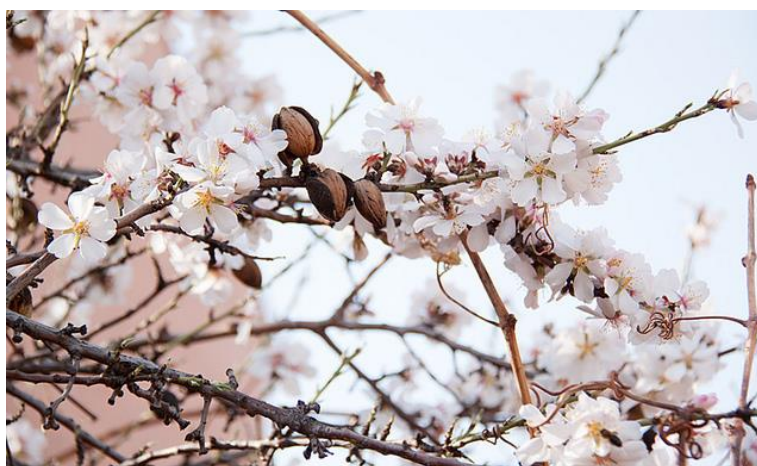


Figure 2. Detail on the flowers and fruits of almond tree.

Almond tree grows best in climates with warm, dry summers and mild, wet winters. The optimal temperature for their growth is between 15 and 30 °C [19]. Almond tree is well adapted to the whole Mediterranean region, from which about 28% of the world production is obtained [13].

However, the world production of almond is still dominated by United States (mainly California) which are responsible for 37.2% of world production. Other important almond producers are Spain (12.1%), Australia (7.4%), Iran (5.2%), Morocco (5.1%), Italy (4.6%), and the Syrian Arabic Republic (4.5%). The remaining production (24.9%) is distributed in other countries, each of them producing <4% of the total [19]. In Portugal, almond is a traditional crop, mainly spread through Algarve and Alentejo in the south, and “Terra Quente Transmontana” in the north [20, 21].

Almond production has increased significantly in recent years, with a worldwide production of about 2.9 million tons in 2013 [22]. Almonds are mostly exported shelled (70%), with the remaining being either unshelled or processed.

These fruits have long been recognized as being commercially valuable and nutritionally important. Freshly harvested almonds are dehulled, and in-shell almonds are dried until the water content is less than 6%. Dried in-shell almonds can be stored for several months before being used in the food industry [23, 24]. Shelled almonds may be sold as whole natural almonds or processed into various almond forms. The whole natural almonds had their shells removed but still retain their brown skins; blanched whole almonds had both their shells and skins removed [25].

Almonds are used in the food industry to produce pastries, cookies (such as the “almendrado” described in the section 1.3), almond syrup, nougat, and marzipan. They are typically used as snack foods and as ingredients in a variety of processed foods, especially in bakery and confectionery products [14]. More recently, almonds are also being processed to make nutritional products such as almond milk, used as a substitute for cow’s milk. In all cases, the quality of nuts is defined particularly by moisture level, lipid content, oil composition, and oil ultraviolet absorption coefficients [20].

Nuts are an important part of the Mediterranean diet. In recent clinical epidemiological studies, diets supplemented with frequent nut consumption, particularly almonds, decreased serum concentrations of low density lipoprotein cholesterol [21, 26, 27] and may protect against coronary heart disease because of their beneficial effects on blood lipids [28, 29]. In fact, the United States Food and Drug Administration (USFDA) allows the following labelling claim: “Scientific evidence suggests but does not prove, that eating 1.5 ounces per day of almonds as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease” [30, 31].

In fact, when used as snacks and in diets of hyperlipidemic subjects, almonds significantly reduce coronary heart disease factors. Moreover, a long term supplementation with almond showed nutrient modification, which closely matched the recommendations to prevent cardiovascular and other chronic diseases [14].

As one of the most popular nut crops, almonds have great significance for human nutrition and health. The high nutritive value of almond kernels arises mainly from their high oil content, which has been reported as containing elevated percentages of monounsaturated fatty acids, especially oleic acid, whereas saturated fatty acids are very low [20].

Despite the chemical variability exhibited by almond cultivars, a comparison among Portuguese, American, Irish, Spanish, Italian, French, Australian and Tunisian samples, allows indicating the following overall values: fat (42-57%), protein (19-23%), carbohydrates (20-27%), fiber (11-15%), moisture (3-9%), ash (2.5-4.5%). Regarding their fat composition, the most abundant fatty acids are oleic acid (50.41-81.20%), linoleic acid (6.21-37.13%), palmitic acid (5.46-15.78%), stearic acid (0.80-3.83%), and palmitoleic acid (0.23-2.52%). Linolenic acid and myristic acid were also detected. Amandin is the main protein in almond, while asparagine (Asn) is by far the most abundant amino acid and sucrose stands out as the predominant sugar [20, 32, 33].

Among its minor compounds, almond contains important vitamin, polyphenols and mineral elements contents. Regarding the vitamins present in almond, vitamin E (especially the α -tocopherol isoform) is the most important, but these nuts are generally recognized as a good source of riboflavin (vitamin B₂) and other complex B vitamins such as thiamine, niacin, pyridoxine, pantothenic acid, folic acid (folate), and biotin. Almonds are also among the top 40 richest food sources of phenolic compounds, mainly present as proanthocyanidins, hydrolyzable tannins, flavonoids, and phenolic acids. The mineral content of almonds, as in other plants, may be affected by many environmental factors and agronomic practices, but their general profile is usually as follows: potassium (K) > phosphorus (P) > calcium (Ca) > magnesium (Mg) > iron (Fe) > zinc (Zn) > manganese (Mn) > selenium (Se) > sodium (Na) > copper (Cu) [20].

The composition in vitamins and phenolic compounds is probably related to some of the biological properties reported for almond extracts: sedative, anti-inflammatory, antimicrobial, anti-hyperlipidemic, antitumoral and antioxidant activities [34, 35]. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary disease and cancer is raising interest among scientists, food manufacturers and consumers since the future trend is toward functional foods with specific health effects [36].

Other recognized benefits of almond consumption include:

- i) helping in the development and health of the human brain, mainly due to their contents in riboflavin and L-carnitine, which have been shown to increase brain activity, resulting in new neural pathways and a decreased occurrence of Alzheimer's disease;

- ii) improving bone health, because due to their contents in many vitamins and minerals that prevent the onset of age-related conditions like osteoporosis;
- iii) promoting heart health, because of their mono-unsaturated fats, protein and potassium, all instrumental in heart health. Vitamin E is an effective antioxidant and also reduces the risk of heart diseases, while the presence of magnesium in almonds can help avoid heart attacks;
- iv) strengthening the immune system, for being great sources for alkali materials, which are known to benefit the strength of the immune system;
- v) exerting anti-inflammatory activity, mainly due to linoleic and linolenic acids that help to reduce inflammation and also help to reduce the levels of LDL(low density lipoprotein) cholesterol;
- vi) favoring the regulation of blood pressure, due to the potassium that helps to regulate blood pressure, and low sodium content;
- vii) boosting energy status, because of the presence of manganese, copper and riboflavin which help in energy production and metabolic rate;
- viii) improving the movement of food through the colon, thereby preventing build-up and potentially colon cancer;
- ix) protecting against diabetes through the reduction of the reactionary rise in glucose and insulin levels after meals, helping to regulate the absorption and processing of glucose, making the entire process much smoother and safer;
- x) helping to reduce the incidence of birth defects in newborn babies so, due to their folic acid contents [37].

However, almonds might also induce some allergies or intolerance. Cross-reactivity is common with nut allergens. Symptoms range from local signs and reactions (e.g., oral allergy syndrome, contact urticaria) to systemic signs and symptoms, including anaphylaxis (e.g., urticarial, angioedema, gastrointestinal and respiratory symptoms) [47].

1.3 ALMOND BY-PRODUCTS

The food processing industry, across the globe, has grown exponentially in the recent years and is still expanding. With the increasing analytical capabilities, we are becoming more aware of the biochemical structures and functions of bioactive compounds in various foods and their effects on the human body [38, 39]. This led to a rise in the popularity of various health promoting functional foods. Functional foods are those similar to conventional food, but with physiological benefits and capacities to reduce the risk of chronic diseases, in addition to their basic nutritional functions [30, 31].

With the advances in nutrition and medical science, it was observed that the non-nutrient components of the food are also very important for maintaining good health and reducing the incidence and risk of common and chronic diseases, in addition to their nutritional function [40].

Consumers all over the world are becoming more conscious of the nutritional value and safety of their food and its ingredients, therefore demanding natural ingredients which are expected to be safe and health-promoting, food that are believed to be safer, healthier and less subject to hazards than their artificial counterparts [41, 42].

The fast growing food processing industry, in the organized sector, especially in the developing world, is expected to generate high volumes of by-products in the near future. A by-product is a substance resulting from a production process, which does not correspond to the primary production objective; *i.e.*, it is not the primary product or service being produced, but a result of another action. In the context of production, this product is the output from a joint production process that is minor in quantity and with low realizable value when compared to the main products [43]. Some of the typical examples of by-products include seeds, skins, pods, peels, pomace, hulls, husks, cores, stones, stems, rinds, or kernels [44].

It is important to make the distinction between materials that are not the main objective of a production process but can be considered as non-waste by-products, and those that should be treated as waste. Food wastes are organic residues from the processing of agricultural raw materials to food, which arise as liquid and solid wastes [40]. A product residue is a material that is not deliberately produced in a production process but may or may not be a waste, while a by-product is a production residue that is not a waste [45]. Many by-products are considered garbage, not being used in any application. Their valorization might boost the development of new products and the fulfilling of sustainability policies. The industry can thus achieve benefits through sustainable management approaches, accompanied by better economic and environmental performance, based on the efficient use of resources, materials and energy [46].

The recovered by-products can be used to produce functional foods and adjuvants to food processing or medicinal and pharmaceutical preparations [40]. Among the potentially marketable components, different high value components such as proteins, polysaccharides, fibers, flavor compounds, and phytochemicals should be exploited as nutritionally and pharmacologically functional ingredients [40].

The by-products of plant food processing represent a major disposal problem for the industry, but those products are also promising sources of compounds which may be used to take advantage of their favorable technological or nutritional properties [47].

The highest ratios of by-products arise from fruits, vegetables and seeds. Due to their increasing production, disposal represents a growing problem since the plant material is usually prone to microbial spoilage, thus limiting further exploitation. On the other hand, costs of drying, storage and shipping of by-products are economically limiting factors [47]. This probably explains why the major fraction of by-products is used for feed production. There is normally a far better profitability in making products for human consumption, which reaches maximum values when used to produce bioactive compounds such as enzymes, peptides, vitamins, biopolymers, phenolic compounds, or sterols, for different applications [48].

As described in the previous section, almonds are one of the world's most nutritious and versatile nuts, renowned for their many health benefits and culinary uses. Their different types of industrial processing generate multiple by-products. The peach-like edible almonds fruit has three distinct parts: the inner kernel or meat, the middle shell portion, and an outer green shell cover or hull. The harvesting procedure starts when the almonds are partly dried on the trees [49]. Once the almond has dried in the field, the hull also dries and begins to separate from the almond. Almond shells are the hard layer between the hull and the almond meat. The shell is what protects the almond from insects while on the tree. Almond shells can be ground up and used as bedding for garden planters and landscape material similar to wood chips. Almond shells are most commonly sold to co-generation plants to be used as a fuel source [50]. Almond hash is the material discarded during the shelling process when there may be nicks or flaws on the meat. These bits and pieces of almonds are separated and used for animal feed. The hash is sold for higher end animal feed than the hulls and can be added to grain feed [50].

Shelled almonds may be sold as whole natural almonds or processed into various almond forms. The whole natural almonds have their shells removed but still retain their brown skins; blanched whole almonds have both their shells and skins removed. Usually, the removed skins are discarded [51].

The external coating of almonds is industrially removed by using a hot water blanching process, with the brown skin contributing around 6.0–8.4% of the seed weight [52, 53].

These different by-products demand implementing new applications, especially considering that their major part is just incinerated or dumped without control (causing several environmental problems), or used as animal feed [54].

As previously indicated, around 12% of the world's almond production is grown in Spain. This leads to the accumulation of large amounts of by products and subsequent environmental problems due to their difficult degradation. On the other hand, natural antioxidants present in almond skins could contribute to the health benefits associated with almond consumption [55].

By estimation, each 1,000 acres of bearing almond orchard can generate more than 4,800 dry tons of biomass annually at hulling and shelling level including pruning and tree removal. While most of the research has been focused on the bioactivity of extracts from almond residues, there might be benefits in using processed almond by-products directly as additives to food products, which would significantly reduce the costs associated with extraction and purification [29].

One of the most up-to-date by-products results from the cold extraction is almond oil. Almond oil is highly appreciated in specialty applications and its commercial value might be as high as 80 €/kg. This elevated cost is also related to the extraction type; when a strictly physical process is adopted (as in the case of the industry that provided the PDAF for this study), the extracted oil has a higher quality. On the other hand, the process is not as efficient as in the case of using chemical solvents. Nevertheless, the almond flour remaining after the extraction process has an elevated quality, since it includes all the bioactive components of almond skins in addition to the high-interest compounds present in the almond oil maintained in the flour.

In general, there are multiple routes to the utilization of almond residues with different purposes. In the present work, the potential utilization of the by-product of almond oil-extraction (almond flour) was thoroughly studied through the development of a novel (and healthier) formulation of a highly appreciated pastry product: "almendrado" (detailed in the next section).

1.4 “ALMENDRADOS”

Biscuits are convenient food products, being very popular among rural and urban populations [44]. Some of the reasons for such wide popularity are their low cost, varied taste, availability and longer shelf life, when compared to other processed foods [56].

Even so, and considering the major aspects focused in the Introduction section, there is a current need to enhance the formulation of these traditional products in order to meet the most recent consumer requirements. The innovation of a traditional product is often based in the identification, evaluation and transfer of knowledge that leads to the implementation of nutritional quality, while maintaining or improving other features recognized by consumers. Besides maintaining the best properties of traditional production, it is aimed to adapt the product to the international market demands. In the particular case of “almendrados” or “amendoados” (**Figure 3**) used in this work, we intend to use an almond by-product to innovate the traditional recipe. The growing interest in high specialty oils extracted from nuts using “clean” extraction procedures is generating increasing amounts of a high-potential by-product: the delipidified flours. In the present work, the almond flour obtained after oil extraction (performed through a cold extraction process conducted in a Spanish company), was used as raw materials to improve the traditional recipe of “almendrados”.



Figure 3. Typical aspect of “almendrados” biscuit.

Besides being considered as a traditional product (since they exist for more than 50 years), “almendrados” were classified with the mention of “Especialidade Tradicional Garantida” (in English: Traditional Specialty Guaranteed - TSG) (**Figure 4**). Specialty refers specifically to a product or foodstuff presenting clearly distinguishable elements, while “traditional” reflects a validated use on the Community market that shows the transmission of knowledge between generations. The traditional specialty guaranteed is in accordance with the Regulation 509/2006 [47].



Figure 4. Portuguese and English versions of the TSG classification to be used in labelling.

The “almendrados” are a popular delicacy throughout Portugal. It is thought that “almendrados” were created in the Algarve region, back in the time when the Arabs occupied the southern region of Portugal. Basically, “almendrados” consist of a biscuit made with almonds, brown sugar, egg whites and a wafer sheet in the bottom. They feature a toasted color aspect, slightly crispy and with a spongy interior, with a flattened round shape [48].

“Almendrados” are specially produced in Spain and Portugal, they are quite versatile and long lasting. Furthermore, these biscuit might be maintained under ease conditions, they are greatly appreciated by general population and, nutritionally, are not considered as a high-calorie sweet, nor detrimental to health.

All the “almendrados” formulations used in this work (except the industrial formulation, which was bought in a grocery store) were professionally prepared in “Confeitaria Luso” and have as ingredients: albumens, almond flour, mixing yellow and white sugar, potato starch, vanilla essence and a very thin wafer layer covering the bottom (please see Annex I for details).

1.5 NUTRITIONAL ANALYSIS

Adequate nutrition is one of the pillars of public health. The estimation of nutrition intake from food consumption requires reliable data in food composition, which is cornerstone of food-based dietary guidelines for healthy nutrition, containing the necessary data regarding specific nutrients in different food sources. Information on food composition is also of great importance for scientists and practitioners working in the field of nutrition and public health. Furthermore, from a food safety and security point of view, nutrition and health claims have to be supported by sound scientific evidence, including data on the food's nutrient content [43].

A nutrient is generally considered as any substance consumed as a constituent of food, which provides energy or is needed for growth and development and maintenance of healthy life or to counteract any deficit that may cause characteristic biochemical or physiological changes in the organism [49]. Thereby, the nutritional value defines what a food product is made of and its impact on the body. Because of disease and weight control, it is particularly important to understand the nutritional value of all food products due to their impact on the body, as it relates to cholesterol, fat, salt and sugar intake. Food label is the primary tool enabling consumers to understand nutritional values in order to make informed decisions about consumption [50].

European consumers are exposed to a wide variety of messages about the relationship between diet and health, and the widespread information about nutrition and health in the press can appear complex or conflicting, not resulting clear to the consumers which is the healthiest choice for a determined food product. Accurate and informative food labelling are important in helping consumers to select the most appropriate foods to be integrated in a healthy balanced diet. However, unclear or misleading information on food products can increase confusion and lead to mistrust of healthy eating messages [57].

While health is valued by everybody and therefore is one of the fundamental drivers of human behavior, attempts to change eating patterns by informing consumers about the link between diet and health have been difficult, although nowadays this concern is more relevant. Nutrition labelling is one of the major instruments in promoting healthier eating patterns. Nutrition labelling is an attempt to provide consumers with information about the nutrition content of individual food products, in order to enable consumers to choose nutritionally appropriate food [58]. It is an attractive instrument for a variety of reasons because it supports the goal of healthy eating, while retaining consumer freedom of choice, and it reduces the need for information search efforts [58].

In all cases, food composition and health claims must be supported by sound scientific evidence, including data on the food's nutrient content [43]. The latter is even more important in the case of bioactive compounds, in light of their varied health effects. Finally, data on contaminants and other potentially harmful compounds are also of public relevance to enable risk assessment [43].

The nutritional value, together with factors such as color, taste, odor, contaminants, adulterants, is among the most important factors contributing towards food quality, thereby constituting one the primary demands of manufacturers and consumers [59].

Proximate analysis of foods refers to determining the major components of foods: moisture, ash (total minerals), fats or lipids, protein and carbohydrates [54].

Moisture is an important factor in food quality, preservation and resistance to deterioration.

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff and represent the total mineral content in foods. Minerals are the constituents remaining as ash after calcination. They may be divided into two categories: main elements (e.g., calcium - Ca, potassium - K, sodium - Na, chlorine - Cl, sodium - Na, magnesium - Mg) and trace elements (e.g., iron - Fe, zinc - Zn, copper - Cu, manganese - Mn, iodine -I). The main elements and number of trace elements are essential because they have specific biological roles. In the same food raw material, the content can vary greatly according to genetic and climatic factors and agricultural procedures [53].

Fat analysis is important for quantitative and qualitative analysis of lipids in foods for accurate nutritional labeling, determination of whether the food meets the standards of authenticity, to ensure that the product meets manufacturing specifications, to evaluate the occurrence of chemical reactions during processing and storage or to predict the true health benefiting properties (for instance, by evaluating their profiles in fatty acids or tocopherols). Fat is also a primary contributor to the palatability of food [9].

Carbohydrates are also important in the analysis of qualitative and quantitative analysis of foods, beverages and their ingredients. Besides ensuring the amounts of specific components of consumer interest, carbohydrates are used to detect adulteration of food ingredients, to control process parameters, to maintain product consistency, or to control the process flow. The consumer desires and market challenges point towards good and healthy foods, available all year around, in sufficient supply, conveniently packaged, presenting long shelf life, maintaining as close as possible the original freshness and an appealing taste [55].

1.6 FATTY ACIDS

For years, we were urged to banish fat from our diets whenever possible, moving towards using low-fat foods. We switched to low-fat foods. But the shift didn't make us healthier, probably because we cut back on healthy fats as well as harmful ones [60].

Human body needs some fat from food because it is a major source of energy and several bioactive compounds (e.g. fat-soluble vitamins). Fat is needed to build cell membranes, the vital exterior of each cell, and the sheaths surrounding nerves, and it is essential for blood clotting, muscle movement, and inflammation response. For long-term health, some fats are better than others: good fats include monounsaturated and polyunsaturated fats and the bad ones include industrial-made trans fats [61].

Lipids are usually defined as those components that are soluble in organic solvents (such as ether, hexane or chloroform), but are insoluble in water. This group of substances includes triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, phospholipids, sterols, carotenoids and vitamins A and D [62].

The majority of lipids are derivatives of fatty acids (FA). Some lipids act as building blocks for biological membranes and occur in food but usually at less than 2% but, even as a minor food constituent, they must receive particular attention, since their reactivity may strongly influence the organoleptic quality. Triacylglycerol's are deposited in several animal tissues and organs of some plants, representing the major source of lipids. After fat extraction by common methodologies, such as the Soxhlet extraction, the analysis of specific lipid molecules, as in the case of FA, is preceded by an hydrolysis step [53].

Saponification reduces the molecular weight and methylation reduces the polarity, both of which increase the volatility of the lipids. The concentration of different volatile fatty acid methyl esters (FAME) present in the sample is then analyzed using gas chromatography (GC). The FAME are dissolved in a suitable organic solvent that is then injected into a GC injection chamber. The sample is heated in the injection chamber to volatilize the FAME and then carried into the separating column by a heated carrier gas. As the FAME pass through the column they are separated into a number of peaks based on differences in their molecular weights and polarities, which are quantified using a suitable detector. Determination of the total FA profile allows calculating the type and concentration of FA present in the original lipid sample [63].

The most abundant FA have straight-chains of an even number of carbon atoms. There is a wide spectrum of chain-lengths, ranging from a four-carbon fatty acid in milk to thirty-carbon fatty acids in some fish oils. Frequently, the fatty acids have eighteen carbons. Double bonds along the carbon chain or substituents are designated chemically by counting the carboxyl carbon as position 1. Thus, the double bonds in linoleic acid give it the chemical systematic name of 9, 12-octadecadienoic acid [64].

Studies show that eating foods rich in monounsaturated fatty acids (MUFA) improves blood cholesterol levels, which can decrease the risk of heart disease. Research also shows that these FA may benefit insulin levels and blood sugar control, which can be especially helpful in type 2 diabetes [65, 66]. MUFA are usually associated with high vitamin E contents (due to its antioxidant properties and lipophilic nature), being the main constituents of almond FA, similarly to the observed in other foods like peanut butter, avocados, seeds and olive, peanut, canola and sesame oils [65].

Polyunsaturated fatty acids (PUFA) are a type of fat found mostly in plant-based foods and oils. Evidence shows that eating foods rich in PUFA improves blood cholesterol levels, which can decrease the heart disease risk, and might have a positive effect against type 2 diabetes [65, 66]. PUFA have more than one double-bonded, or unsaturated carbon atom, and are mainly found in soybean oil, corn oil, safflower oil, salmon, trout, mackerel, herring, walnuts and sunflower seeds [65].

PUFA can be divided into two categories: omega-6 FA and omega-3 FA. Omega-3 FA play a role in normal growth and development and proper brain function, they also reduce widespread inflammation and decrease the risk of chronic diseases, such as heart disease, arthritis and cancer. Like omega-3 FA, omega-6 FA play an active role in growth, development and brain function, but they also regulate metabolism, stimulate hair growth and keep the reproductive system healthy [67].

Considering the essential roles of MUFA and PUFA, it is important to accurately determine the total fat content and the FA profile, which might also be good quality indicators of the correct processing of foods.

1.7 VITAMIN E

Vitamins are organic substances present in several foods in low quantities and are indispensable to organism functions. Its systematic absence in the diet can result in deficient growing and development [68].

Independently of environmental factors, animals cannot, usually, synthesize vitamins by anabolic pathways, and, for this reason, it is necessary to include vitamins in the diet. In general, the vitamins are necessary in micro quantities and dietary doses vary with age, physiological state and physic activity. The nutritional necessity for vitamins increases in the growing, pregnancy and lactation periods, in condition of intensive work and during the occurrence of diseases, mainly the infectious one [69].

Traditionally vitamins are classified in fat-soluble (A, D, E and K) and hydro soluble (C and B complex: B1, B2, B3, B5, B6, B8, B9 and B12) [70]. The correlation between diet and health led the consumers to ingest foods containing vitamins, included the fortified ones, and more recently, the pharmaceutical supplements. Due to the nutritional importance of vitamins, several analytical methodologies have been developed for determination of these substances in food, pharmaceutical supplements and biological fluids [71].

There multiple analytical procedures to carry out the determination of vitamins in food, pharmaceutical and physiological samples such as chromatography, spectrophotometry, voltammetry and spectrophotofluorimetry [68]. The chromatographic method is one of the most important to carry out determination of vitamins as its exploitation permits to separate the analytes before the detection [71].

The most commonly used methods of sample preparation for the analysis of fat-soluble vitamins in foods and pharmaceuticals include sample saponification and later extraction of vitamins from the unsaponifiable matter into an organic solvent, and separation by normal- or reversed-phase high-performance liquid chromatography (HPLC) coupled to ultraviolet (UV) or fluorescence detection. [72, 73]. Alternatively, they might be promptly obtained by direct solvent extraction of the vitamins from the sample [74].

The organic solvents are usually hexane, diethyl ether, ethyl acetate, toluene, or mixtures thereof. The obtained extracts can then be used for analysis by normal-phase HPLC, either directly or after additional concentration and/or cleanup steps [75].

Saponification or alkaline digestion readily causes oxidation of fat-soluble vitamins although some investigators have tried to overcome this obstacle by adding antioxidants such as butylated hydroxyl toluene and ascorbic acid [76]. Saponification procedures might also introduce large variation and have low recovery and reproducibility, in addition to being time-consuming [68].

Fat-soluble vitamins have important roles in several functions of the human body, as they regulate various processes such as vision (Vitamin A), calcium absorption (Vitamin D), antioxidant protection in cell membranes (Vitamin E), and blood coagulation (Vitamin K) [70, 77, 78]. These vitamins are substances often found associated in food and pharmaceutical products [68]. The widespread use of this kind of food and pharmaceutical supplements demands simple, fast and reliable methods for the determination of active compounds in commercial forms [77]. For example, the lack of vitamin E may lead to breakage of cell membranes, possibly leading to heart diseases and certain cancers.

Vitamin E is a collective term for tocopherols and tocotrienols [74] and is found in foods in eight forms: α (alpha), β (beta), γ (gamma) and δ (delta) tocopherols and the four analogous tocotrienols. The proportions of each vitamer might be affected by the geographical origin, storage temperature and age of the food product [79]. The main lipophilic antioxidant is α -tocopherol, which inhibits peroxidation of PUFA in cell membranes. It is a fat-soluble vitamin with antioxidant capacity, mainly due to its capacity to react with free radicals [80]. This is particularly relevant, since high concentrations of antioxidants are associated with a reduction in the risk of disorders connected to free radicals such as cancer, atherosclerosis and cell damages. In fact, a reduction in the risk of coronary illness as a result of a high intake of vitamin E has been indicated [81] .

Vitamin E is not synthesized within the body and, as essential to our organism, must be supplied through the diet [75]. Since fat soluble vitamins are sensitive to oxygen, light, heat and extreme pH values, the fortification of food is commonly achieved with the more stable vitamin esters, such as retinol acetate and tocopherol acetate [82].

1.8 SENSORY ANALYSIS

Product developers make use of many tools in the development of a product. These tools include, for example, chemical tests, microbiological procedures and the use of physical equipment to determine elasticity, hardness, viscosity, color, intensity, etc. [76]. Even so, it is nearly impossible for food products to present the same exact measurements or results when these tools are applied individually. Furthermore, the food products are still affected by the different consumer's perceptions, acceptability or preferences [76].

Grading methods for food and beverages products, traditionally involve one or two trained "experts" assigning quality scores on the appearance, flavor and texture of the products based on the presence or absence of predetermined parameters. These traditional judging methods have several shortcomings because they can't predict consumer acceptance, making their quality assessments quite subjective [77]. Thus, by using traditional methods of evaluation, some products with very different sensory characteristics, such as those identified by a product flavor profile, but with no product defect, might obtain the same quality score [76]. This is where sensory evaluation becomes an invaluable tool. Users of products, experience them with their natural senses and not with equipment or laboratorial tests [76].

A broad spectrum of sensory characteristics, including appearance, aroma, flavor, and texture are used by consumers to make purchasing and consumption decisions related to foods [83]. In order to improve the quality of their products, food companies have a need for sensory expertise [84]. Initially, this need was fulfilled by hiring and training a limited number of sensory experts, but with the development of sensory evaluation methods and statistical techniques in the 20th century, the task of the sensory expert was progressively taken over by laboratory panels of trained employees or groups of untrained consumers [85].

This approach has its limitations for understanding food perception, because in everyday situations food perception depends on the contextual conditions and the way the food is consumed, and there is a need to extend sensory investigations beyond the properties of the food itself. For instance, due to a trend towards increasing consumer convenience, more and more foods tend to be available in single portion size packages from which they can be consumed directly (e.g., desserts, cookies, salads) [84] and, furthermore, food products may be consumed in many different settings. Consumers are influenced by the characteristics of the environment and by any information they have obtained previously on the product [86]. As a consequence, methods of sensory evaluation are now increasingly being used in research on non-food products.

Sensory evaluation is defined as a scientific method used to evolve, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing [85]. Depending upon the research question, sensory food science utilizes physicochemical, physiological, and consumer-based research methods. The importance of sensory food science is based on the relevance of consumer perceptions to the acceptance and commercial success of foods and on the significance of food for human well-being and health. In food companies, sensory food science can be of great value to both tactical and strategic research goals [86].

So, sensory food science is an experimental method of food analysis which makes use of human senses as instruments [87] and provides unique information about the degree of acceptance of a food and it is also widely utilized for the determination of overall quality [87] and can be a method that allow the consumer to perceive significant differences between processed products in different ways, evaluating and determining the acceptance of the product in the market [88].

It can be considered to be an interdisciplinary science that uses human panelist's sensory perception related to thresholds of determination of attributes, the variance in individual sensory response experimental design to measure the sensory characteristics and the acceptability of food products [79].

Sensory tests provide useful information about the human perception of product changes due to ingredients, processing, packaging, or shelf life. Sensory analysis is not new to the food industry, but their application as a basic tool in food product development and quality control has not been given the recognition and acceptance it deserves. Similar to other industrial products, food products available in highly saturated markets should not only provide good quality and be appealing, but they should offer interesting and engaging experiences in order to seduce consumers to purchase them [84].

Sensory evaluation can be used to compare similarities/differences in a range of products, analyze food samples for improvements, gauge responses to a product, explore specific characteristics of an ingredient, check whether a final food product meets its original specification and provide objective and subjective feedback data to enable informed decisions to be made. It is based on fundamental psychological perception and physiological techniques [89] and hedonic scales are used by experts and untrained consumers, since the best results are often obtained using untrained panels [90].

Sensory assessment may be made by three types of assessor: "assessors", "selected assessors" or "expert assessors". Assessors can be "naive assessors" who do not have to meet a precise criterion of selection or training, or people who have already taken part in some sensory tests (initiated assessor) [91].

The nature of the product to be tested determines the experimental protocol of the test, and may also have an influence on the type of test that is required to satisfy the test objectives. There are many types of sensory analysis methods, the most popular being discrimination tests (used to determine the probability of difference or similarity between products), descriptive tests (used to identify the specific sensory attributes present in a sample) and consumer acceptance tests [79]. Consumer acceptance, preference and hedonic tests are used to determine the degree of consumer acceptance for a product. Samples of food should be uniform in size and of the same temperature at serving. They should be coded by a random three-digit number and presented in clean odor-free containers [76]. Palate cleansers may be used by the assessors between samples and between sessions, but care should be taken to ensure that they do not influence the flavor of products to be assessed [91].

If we want to know if some products are different, the best sensory evaluation method is to use difference or discriminatory testes, using 20 to 50 tasters. If we want to know what is the acceptability of a product or if is one product preferred over another, we should use hedonic tests with 70 – 150 consumers and make a screed for product use with questions such as “Do they buy the product?”, “How often?”, asked degree of liking and preference questions [76].

Without sensory evaluation, development efforts reflect the personal feelings, views and choices of the product developer, product development team, marketer and top management. Thus, without evaluation results useful to the base product development, trade-offs and decisions, product development successes will be few and development timelines very long [76]. Instrumental, physiochemical and sensory analyses are used to evaluate intrinsic characteristics of the physical product, such as odor, taste, size or appearance. For food and beverage products, sensory analyses are the main concepts of integration with marketing where the priority is on people's perceptions of sensory quality, rather than the real taste evaluations. In order for players in the food and beverage industry have a market success, they should ensure that the quality of food is appealing and appetizing or more specifically that the eating quality attributes of aroma, taste, tactual properties and appearance are acceptable to the consumer so that they desire for more.

2. MAIN OBJECTIVE

The main purpose of this study was the development of an enhanced formulation of a traditional and highly appreciated almond-base cookie: “almendrado”. Besides its high acceptability, the selected product is not considered as a high-caloric food, nor regarded as having deleterious health effects.

To a complete assessment of the developed formulations, the proximate composition, fatty acids and tocopherols profiles were thoroughly studied and compared among i) industrial “almendrados” purchased at a grocery store (AI); ii) “almendrados” produced according to the traditional recipe (AT); iii) “almendrados” produced according to the traditional recipe added with PDAF (AF); and iv) “almendrados” produced according to the traditional recipe added with PDAF and with a 30% sugar reduction (AFSR). Likewise, the consumer’s acceptability of all prepared formulations was evaluated using a sensory analysis performed by a group of 74 tasters that filled up a 10 parameter questionnaire based in a 7-level hedonic scale for each question (except in one case, where a 5-level scale was used).

2.1 SPECIFIC OBJECTIVES

Throughout the development of the work the following specific objectives were also considered:

- Determination of the nutritional composition (moisture, ash, fat, protein and carbohydrate) of each “almendrado” formulation;
- Assessing any potential change in fatty acids and vitamin E profiles;
- Evaluating differences in the sensorial perception of previously available and newly developed formulations of “almendrados”;
- Implementing a new application for an otherwise useless by-product, thereby stimulating sustainable approaches;
- Perceiving the acceptability of a food product incorporating an industrial by-product as well as the tolerability of modifying a traditional recipe.

3. MATERIAL AND METHODS

This chapter describes the methodologies used in the evaluation of the nutritional composition, determination of lipid compounds and sensory analysis.

3.1 STANDARD AND REAGENTS | MATERIAL AND EQUIPMENT'S

All standards and reagents were of analytical purity “proanalysis” and are presented in the following list:

Determination of protein content:

- Kjeldahl tablets catalyst ($\text{Na}_2\text{S}_2\text{O}_8/\text{CuSO}_4$) and sulfuric acid (H_2SO_4) concentrated 96% of Merck (Darmstadt, Germany);
- Sodium hydroxide (NaOH) of VWR International (Leveun, Belgium); boric acid 4% (H_3BO_3), Panreac (Barcelona, Spain).

Determination of total fat:

- Anhydrous sodium sulfate (Na_2SO_4) of Merck (Darmstadt, Germany);
- Petroleum ether from Sigma Chemical Co. (St. Louis, USA).

Determination of the total content of Vitamin E:

- *n*-Hexane HPLC of Merck (Darmstadt, Germany);
- 1,4-Dioxane from Sigma Chemical Co. (St. Louis, USA);
- Tocopherols and tocotrienols standards: α -, β -, γ -, δ -tocopherol and α -, β -, γ - and δ -tocotrienol from Calbiochem (La Jolla, CA, USA);
- Tocol internal standard: 2-methyl-2-(4,8,12-trimethyl tridecyl)-chroman-6-ol from Matreya Inc. (PA, USA);
- Absolute ethanol of Fisher Chemical (Loughborough, England);
- Anhydrous sodium sulfate (Na_2SO_4), Merck (Darmstadt, Germany);

Determination of fatty acid profile:

- Methanol of VWR International (Leuven, Belgium);
- Potassium hydroxide (KOH) from Panreac (Barcelona, Spain);
- *n*-Hexane HPLC and anhydrous sodium sulfate (Na_2SO_4), Merck (Darmstadt, Germany);
- Mixture of fatty acid standards (FAME 37, Supelco, Bellefonte, PA, USA).

3.2 SAMPLING

The “almendrados” manufactured in “Confeitaria Luso”, in Oporto, were collected on the day of production and stored in cardboard boxes that allowed them to be kept fresh and fluffy for the maximum possible time.

The samples used in this study were grouped as mentioned below:

- 1) “Almendrados” bought at a local grocery: AI;
- 2) “Almendrados” produced at “Confeitaria Luso”: AT;
- 3) “Almendrados” produced at “Confeitaria Luso” added with PDAF: AF;
- 4) “Almendrados” produced at “Confeitaria Luso” added with PDAF and with a 30% sugar reduction: AFSR (**Figure 5**).



Figure 5. “Almendrados” prepared at “Confeitaria Luso” facilities.

The different samples were ground in a mill (GM Grindomix 200, Retsh, Germany) (**Figure 6**) for 20 seconds at a speed of 3000 rpm and for 20 seconds at speed 5000 rpm to obtain a fine powder. Then, samples were homogenized (**Figure 7**) and properly stored at -20 °C protected from light.



Figure 6. Equipment used to grind “almendrados” samples (GM Grindomix 200, Retsh).



Figure 7. Grinded and homogenized “almendrados” samples.

3.3. NUTRITIONAL ANALYSIS

Moisture, protein, fat and ash content were determined accordingly to the AOAC procedures [92]. Protein content was determined by Kjeldahl method and fat by Soxhlet extracting method, whereas ash content was determined by incineration. Carbohydrates were calculated by difference. All proximate composition analyses were done in triplicate.

3.3.1 MOISTURE QUANTIFICATION

Moisture content was determined using a scale equipped with an infrared lamp (Model SMO Scaltec® 01, Scaltec Instruments, Germany) (**Figure 8**). A portion of proximately 5 g was weighed and dried at 105 ± 2 °C till constant weight. The loss in weight was used to calculate the water content. Results were expressed as g/100 g fresh sample.



Figure 8. Moisture analyzer (Scaltec SMO 01).

3.3.2 ASH QUANTIFICATION

The determination of total ash content was determined by direct incineration in a muffle furnace (**Figure 9**). It was weighed approximately 2 g of sample that was placed in a muffle furnace (Thermolyne 48000, F48010-26, Electrothermal Engineering Ltd, Essex, UK), heated gradually (every 30 minutes, 50 °C) till $500-550 \pm 15$ °C and maintained for 12 h, following the official method of AOAC (association of analytical communities) [93]. The white ash content was determined by the weight difference before and after the incineration process.

The analyses were performed in triplicate for each sample and the results expressed as g/100 g of sample (fresh weight).



Figure 9. Muffle furnace.

3.3.3 PROTEIN CONTENT QUANTIFICATION

The determination of protein content was performed by the Kjeldahl (**Figure 10**) method [94] by quantification of total nitrogen in the samples. About 1 g of sample, weighed in a nitrogen-free paper, was placed in a Kjeldahl tube, along with two Kjeldahl tablets (free of selenium and mercury) and 20 ml of sulfuric acid (H_2SO_4 , 96%). Acid digestion was performed in an automatic digester K-438 (Buchi®, Buchi Labortechnik AG, Switzerland) where all organic material has been destroyed by oxidation so that the organic nitrogen originated ammonium salts. The vapors were neutralized by a gas purifying system (Scrubber B-414 (Buchi®, Buchi Labortechnik AG, Switzerland).

Then, after the alkalization with 90 ml of sodium hydroxide (NaOH , 32%), it is released ammonia. The ammonia was collected in the distillation K-360 automatic distillation unit (Buchi®, Buchi Labortechnik AG, Switzerland) in 60 ml of boric acid (H_3BO_4 4%; pH 4.65) according to the manual Buchi Labortechnik AG, 2007. The result of digestion was titrated with H_2SO_4 (0.1 M) using as an indicator methyl red.

The conversion factor of the total nitrogen used was 6.25 [95].

The analysis were performed in triplicate for each sample and the results expressed as g/100 g (fresh weight).



Figure 10. Kjeldahl equipment.

3.3.4 TOTAL FAT QUANTIFICATION

Fat content was determined by Soxhlet (**Figure 11**) method [96]. Briefly, anhydrous sodium sulfate (Na_2SO_4) was added to ≈ 5 g of sample to retain any residual humidity and treated sand to prevent clogging the passage of solvent. The mixture was transferred to cellulose cartridges and placed into Soxhlet extraction ampoules. The extraction was carried out at 40-60 °C during 8 hours with petroleum ether. After the extraction, the solvent was removed and the fat content was determined. For that, the fat extracted was put in an oven at 105 °C for periods of 30 minutes until constant weight.

The analyses were performed in triplicate and the results expressed as g/100 g (fresh weight).



Figure 11. Soxhlet equipment.

3.3.5. CARBOHYDRATES QUANTIFICATION

The carbohydrate content was calculated indirectly as the difference of the remaining parameters of the nutritional profile of the samples according to the following equation:

$$\% \text{ Carbohydrates} = 100 \% - (\%_{\text{protein}} + \%_{\text{fat}} + \%_{\text{ash}} + \%_{\text{water}})$$

The results were presented as g/100 g (fresh weight).

3.4 VITAMIN E DETERMINATION

3.4.1 SAMPLE PREPARATION

In a centrifuge tube it was weighed ≈ 0.5 g of sample. Then, 10 ml of *n*-hexane, 20 μ l of internal standard (1 mg/ml) and 5 ml of absolute ethanol were added. Samples were vortexed (VWR International) and left under constant agitation for 30 minutes on a stir plate (MS-H-S10, DragonLab). Thereafter, it was added 5 ml of 1% NaCl (sodium chloride) solution. The mixture was vortexed and centrifuged (Thermo Scientific, Heraeus Megafuge 16, USA) at 5000 rpm for 2 minutes. The organic phase was separated and the supernatant was transferred to a falcon tube. The residue was re-extracted three times with 10 ml of *n*-hexane. After each centrifugation, the supernatant was collected. The organic phases were combined and a sufficient amount of anhydrous sodium sulfate (Na_2SO_4) was added to eliminate any remaining water [97].

The mixture was vortexed and centrifuged in order to collect the *n*-hexane layer. The extract was taken to dryness under a nitrogen stream (**Figure 12**) (Sample Concentrator block heater BH200D/3), at room temperature. The residue obtained was reconstituted with 1 ml of *n*-hexane and stored (-20 °C) till further analysis. All extractions were performed in amber glassware in order to be protected from light.



Figure 12. Nitrogen stream device.

3.4.2 CHROMATOGRAPHIC ANALYSIS

Vitamin E content was determined on the extracted fat obtained by cold extraction method with minor modifications [97]. For the identification of individual compounds, standard solutions were prepared, containing the following vitamers: α -, β -, γ - and δ -tocopherol and α -, β -, γ - and δ -tocotrienol. Each of these solutions contained 20 μ L of the internal standard tocol (2-methyl-2-(4,8,12- trimethyl tridecyl) chroman-6-ol) at a concentration of 1 mg/ml.

Chromatographic analysis was carried out in a HPLC integrated system (Jasco, Tokyo, Japan) equipped with an automated injector (AS-2057), a pump (PU-2089) and a multiwavelength diode array detector (DAD) (MD-2018) and a fluorescence detector (FD) (FP-2020).

Chromatographic separation of the compounds was achieved on a normal phase SupelcosilTM LC-SI column (3 μ m; 75 mm \times 3.0 mm; Supelco, Bellefonte, PA, USA) operating at constant room temperature. The injection volume was 20 μ l, eluted with 1.8% dioxane in *n*-hexane (v/v) at a flow rate of 0.8 ml/min.

The compounds were identified according to their UV/vis spectra and by the comparison of their retention time with those of the standards.

Compounds quantification was performed by the internal standard method using the chromatograms obtained with the fluorescence detector response (excitation at 290 nm and emission at 330 nm) of each standard converted to concentration units through the calibration curves [98]. Calibration curves were obtained by preparing a standard stock solution containing individual compounds (α -, β -, γ - and δ -tocopherol and α -, β -, γ - and δ -tocotrienol) in *n*-hexane, subsequently diluted (25 - 1.25 μ g/ml). Each solution contained 20 μ l of the internal standard tocol (1 mg/ml).

Data were analyzed with JASCO-ChromNAV software (version 1.18.03; Jasco, Tokyo, Japan).

3.5 FATTY ACIDS DETERMINATION

For the extraction of the samples lipidic fraction for fatty acids determination it was used the methodology described in 3.4.2.

3.5.1 DERIVATIZATION

After proper sample preparation, ≈ 40 μl of oil were mixed in a clear vial, with 2 ml of *n*-hexane. To this solution it was added 200 μl of a methanolic solution of potassium hydroxide 2 M and vortexed for 1 minute. Then, it was added a sufficient amount of anhydrous sodium sulfate (Na_2SO_4) to remove traces of water. Later, the solution was vortexed and centrifuged (Thermo Scientific, Heraeus Megafuge 16, USA) at 3000 rpm for 5 minutes [99]. The supernatant was transferred to an injection vial and stored at -20 $^{\circ}\text{C}$ until chromatographic analysis.

3.5.2 CHROMATOGRAPHIC ANALYSIS

Fatty acid methyl esters (FAME) separation was performed in a gas chromatograph (GC) GC-2010 *Plus* (Shimadzu, Tokyo, Japan) coupled with a split/splitless Shimadzu AOC-20i auto-injector (Shimadzu, Tokyo, Japan) and with a flame ionization detector (FID) (Shimadzu, Tokyo, Japan). A CP-Sil 88 silica capillary column for FAME (50 m x 0.25 mm i.d, 0.20 μm film thickness; Varian, Middelburg, Netherlands) was used. Helium was the carrier gas at a flow rate of 40 ml/min and the temperature program was as follows: 120 $^{\circ}\text{C}$, for 5 min, programmed to increase to 220 $^{\circ}\text{C}$ at 3 $^{\circ}\text{C}$ per min, and a constant temperature of 220 $^{\circ}\text{C}$ during 10 min; injector and detector temperatures were 250 and 270 $^{\circ}\text{C}$, respectively; run time, 48.33 min. A split ratio of 1:25 was used and the injection volume was 1.0 μl .

FAMES were identified by comparison with the retention times of standard mixtures (FAME 37, Supelco, Bellefonte, PA, USA). Data were analysed using the Shimadzu software GC Solution (Shimadzu, Tokyo, Japan) based on the relative peak areas.

The results were expressed in relative percentage of each fatty acid.

3.6 SENSORY ANALYSIS

The sensory evaluation of the prepared “almendrados” was carried out mainly in a public space of “Confeitaria Luso”, located in the center of Porto. Some additional, sensory test were conducted at the Laboratory of Bromatology, Faculty of Pharmacy, University of Porto.

Methods of preparation and presentation of samples were appropriate for the product and to the proposed questionnaire, following the recommendations of ISO 16820:2004 and ISO 6658:2005.

All the participants accepted voluntarily to do the evaluation and also to be involved in the study. Evaluation was conducted during three different days, using a hedonic scale. A total of 74 participants evaluated the organoleptic properties of the four “almendrados” types, each identified with a three-digit number randomly generated by a computer program. A glass of water (neutral, tasteless and odorless) at room temperature was also available for tasters and they were asked to swallow samples and to rinse their mouths with water to cleanse their palate between samples [100].

It was required to tasters to analyze sensory samples and give notes according to the intensity of preference. The values were recorded on the card given to them for further evaluation. Samples were submitted to acceptability tests and were evaluated by the 74 non-trained tasters. Participants were asked to evaluate products attributes as appearance, taste, sweetness, crunchiness, and hardness. Overall quality, acceptability and also the buying attitude and preference order were evaluated. The ratings were on a 7-point hedonic scale, ranging from 1 (“disliked very much”) to 7 (“liked very much”) for each attribute. For the buying attitude, the scale ranged from 1 (“certainly not buy”) to 5 (“certainly buy”).

The document used for sensory analysis is presented in the following pages.

FICHA DE ANÁLISE SENSORIAL**Idade:**☐ 13-20 ☐ 21-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ 61-70 ☐ ≥ 71 **Sexo:** ☐ Feminino ☐ Masculino**Aproximadamente com que frequência costuma consumir almendrados?**☐ Muito frequentemente ☐ Frequentemente ☐ Ocasionalmente
☐ Raramente ☐ Nunca**ANTES DA DEGUSTAÇÃO**

(assinale com um X na quadricula que mais se enquadra na sua análise)

1. Aspeto geral

1) Desgostei muito; 2) Desgostei; 3) Desgostei ligeiramente; 4) Indiferente; 5) Gostei ligeiramente; 6) Gostei; 7) Gostei muitíssimo;

742

1	2	3	4	5	6	7
---	---	---	---	---	---	---

331

1	2	3	4	5	6	7
---	---	---	---	---	---	---

826

1	2	3	4	5	6	7
---	---	---	---	---	---	---

104

1	2	3	4	5	6	7
---	---	---	---	---	---	---

DURANTE A DEGUSTAÇÃO

(assinale com um X na quadricula que mais se enquadra na sua análise)

1. Sabor

1) Desgostei muito; 2) Desgostei; 3) Desgostei ligeiramente; 4) Indiferente; 5) Gostei ligeiramente; 6) Gostei; 7) Gostei muitíssimo;

742

1	2	3	4	5	6	7
---	---	---	---	---	---	---

331

1	2	3	4	5	6	7
---	---	---	---	---	---	---

826

1	2	3	4	5	6	7
---	---	---	---	---	---	---

104

1	2	3	4	5	6	7
---	---	---	---	---	---	---

2. Intensidade do doce

1) Desgostei muito; 2) Desgostei; 3) Desgostei ligeiramente; 4) Indiferente; 5) Gostei ligeiramente; 6) Gostei; 7) Gostei muitíssimo;

742

1	2	3	4	5	6	7
---	---	---	---	---	---	---

331

1	2	3	4	5	6	7
---	---	---	---	---	---	---

826

1	2	3	4	5	6	7
---	---	---	---	---	---	---

104

1	2	3	4	5	6	7
---	---	---	---	---	---	---

3. Crocante

1) Desgostei muito; 2) Desgostei; 3) Desgostei ligeiramente; 4) Indiferente; 5) Gostei ligeiramente; 6) Gostei; 7) Gostei muitíssimo;

742

1	2	3	4	5	6	7
---	---	---	---	---	---	---

331

1	2	3	4	5	6	7
---	---	---	---	---	---	---

826

1	2	3	4	5	6	7
---	---	---	---	---	---	---

104

1	2	3	4	5	6	7
---	---	---	---	---	---	---

4. Dureza

1) Desgostei muito; 2) Desgostei; 3) Desgostei ligeiramente; 4) Indiferente; 5) Gostei ligeiramente; 6) Gostei; 7) Gostei muitíssimo;

742

1	2	3	4	5	6	7
---	---	---	---	---	---	---

331

1	2	3	4	5	6	7
---	---	---	---	---	---	---

826

1	2	3	4	5	6	7
---	---	---	---	---	---	---

104

1	2	3	4	5	6	7
---	---	---	---	---	---	---

APÓS DEGUSTAÇÃO

(assinale com um X na quadricula que mais se enquadra na sua análise)

1. Qualidade global

1) Desgostei muito; 2) Desgostei; 3) Desgostei ligeiramente; 4) Indiferente; 5) Gostei ligeiramente; 6) Gostei; 7) Gostei muitíssimo;

742

1	2	3	4	5	6	7
---	---	---	---	---	---	---

331

1	2	3	4	5	6	7
---	---	---	---	---	---	---

826

1	2	3	4	5	6	7
---	---	---	---	---	---	---

104

1	2	3	4	5	6	7
---	---	---	---	---	---	---

2. Atitude de compra

1) Certamente não compraria; 2) Provavelmente não compraria; 3) Tenho dúvidas se compraria; 4) Provavelmente compraria; 5) Certamente compraria;

742

1	2	3	4	5
---	---	---	---	---

331

1	2	3	4	5
---	---	---	---	---

826

1	2	3	4	5
---	---	---	---	---

104

1	2	3	4	5
---	---	---	---	---

3. Após degustação de todas as amostras, ordenar por preferência.

(Colocar o número no quadrado pela ordem correspondente)

Menos Preferível

--	--	--	--

 Mais preferível

Muito Obrigada.

Figure 13. Sensory analysis sheet.

3.7 STATISTICAL ANALYSIS

For each formulation, three independent samples were analysed and each sample was analysed in triplicate. Data were expressed as mean \pm standard deviation. All statistical tests were performed at a 5% significance level using IBM SPSS Statistics for Windows, version 22.0. (IBM Corp., USA).

An analysis of variance (ANOVA), followed by Tukey's test (homoscedastic distributions) or Tamhane's T2 test (heteroscedastic distributions) was used to classify the statistical differences among "almendrados" formulations in each of the assayed parameters. The fulfillment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively.

4. RESULTS AND DISCUSSION

In order to compare the different “almendrados” formulations the proximate composition, tocopherols and fatty acids profiles were characterized. The detected differences were statistically classified by the Tukey’s HSD or the Tamhane’s T2 tests, depending on the fulfillment of the homogeneity of variances criterion.

The main purpose of this set of analysis was verifying any potential improvement obtained through the incorporation of the PDAF, that way scientifically validating the enhancement of the traditional recipe.

4.1 NUTRITIONAL ANALYSIS

Table 1 shows the mean values, presented in g/100 g of fresh weight (fw), obtained for the proximate composition of the analyzed formulations. The most obvious differences were observed among the commercial “almendrados” (AI), which showed higher fat content (30 ± 1 g/100 g dw) and lower levels of protein (3.7 ± 0.2 g/100 g dw), carbohydrates (64 ± 1 g/100 g dw) and water (1.6 ± 0.1 g/100 g dw). The considerable difference in the fat content might be explained by the inclusion of lard in the AI (Annex I). Nevertheless, the major component in all formulations were the carbohydrates, followed by fat or protein, water and ash. Among the formulations prepared at “Confeitaria Luso”, the “almendrados” exclusively prepared for this study (AF and AFSR) showed significantly higher contents in mineral elements (1.4 g/100 g dw) than the traditional formulation (0.82 ± 0.05 g/100 g dw). The contents in fat and protein also showed statistically significant differences, with AT presenting the highest values (12 ± 1 g/100 g dw) of fat, while AFSR gave the highest protein content (13.9 ± 0.5 g/100 g dw). No statistical differences were found regarding the carbohydrates contents.

The increase in the protein contents observed in comparison to the AT might be explained by the incorporation of the PDAF in AF and AFSR, since this product has a high protein content. The fat content of this by-product should also be highlighted, since nearly 50% of the fat present in almonds [101] were maintained in almond flour after extraction. Except for the fat content, the nutritional profiles of PDAF is in agreement with the reported for whole almonds [102, 103].

Besides the indicated improvements, the caloric value of the new prepared formulations is an additional positive point, since AF and AFSR the lowest energy values (407 ± 2 and 400 ± 3 kcal/100 g dw, respectively).

Table 1. Proximate composition (g/100 g dry weight) and corresponding energy (kcal/100 g fresh weight) for the prepared “almendrados” formulations. Values for the almond flour alone are also presented to serve as reference. The results are presented as mean \pm SD.¹

		Water	Fat	Protein	Carbohydrates	Ash	Energy (kcal)
Formulation	Al	1.6 \pm 0.1 d	30 \pm 1 a	3.7 \pm 0.2 d	64 \pm 1 c	0.81 \pm 0.03 c	523 \pm 4 a
	AT	3.3 \pm 0.2 c	12 \pm 1 b	9.1 \pm 0.3 c	74 \pm 1 a	0.82 \pm 0.05 c	427 \pm 3 b
	AF	4.2 \pm 0.1 b	10 \pm 1 c	10.4 \pm 0.5 b	75 \pm 1 a	1.4 \pm 0.1 b	407 \pm 2 c
	AFSR	5.4 \pm 0.3 a	10 \pm 1 c	13.9 \pm 0.5 a	69 \pm 1 b	1.7 \pm 0.2 a	403 \pm 3 c
Homoscedasticity ²	<i>p</i> -value (n = 36)	<0.001	0.452	0.007	0.360	<0.001	0.465
One-way ANOVA ³	<i>p</i> -value (n = 36)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PDAF		7.1 \pm 0.2	27 \pm 1	43 \pm 1	18 \pm 1	5.5 \pm 0.3	478 \pm 3

¹Differences among means were evaluated using the Tukey's HSD (homoscedastic distribution) or the Tamhane's T2 (heteroscedastic distribution) multiple comparison tests.

²Homoscedasticity among formulations was tested by means of the Levene test: homoscedasticity, *p*-value>0.05; heteroscedasticity, *p*-value<0.05.

³*p*<0.05 meaning that the mean value of the evaluated parameter of at least one formulation differs from the others (in this case multiple comparison tests were performed).

4.2 VITAMIN E

Table 2 shows the mean values obtained for tocopherols and tocotrienols profiles of each “almendrado” formulation. Considering the utilized ingredients, the main source of vitamin E is almond (or almond flour). Therefore, it was expectable having α -tocopherol as the main vitamer, since it represents more than 90% of all vitamin E isoforms present in almond [101, 104, 105].

The profiles obtained for AT, AF and AFSR are in general agreement with the previous consideration, but that characterized for AI is clearly shows that other ingredients should have used in addition to those defined in the traditional recipe. This hypothesis is validated by the labelled information in AI (Annex I), from which it might be easily concluded that the lard included in the formulation should be the ingredient that justifies the differences in vitamin E profiles, especially the higher contents of γ - and δ -tocopherol and the exclusive presence of β -tocotrienol.

Furthermore, the values obtained for AI are also increased by the fat percentage, since the results are presented in mg/100 g dw.

Among the “almendrados” prepared in “Confeitaria Luso”, AT gave the highest α -tocopherol content (4.9 ± 0.2 mg/100 g dw), probably due to their high content in powdered almond, which contains nearly 60% of fat [101], in comparison to the 27% presented by the PDAF. The differences measured for the remaining vitamers were less pronounced, especially for γ - and δ -tocopherol, which did not show statistically significant differences among the “almendrados” prepared in “Confeitaria Luso”.

In terms of total vitamin E contents, AI showed the highest values (7.2 ± 0.3 mg/100 g dw), mainly due to the already explained reasons, followed by AT (5.3 ± 0.2 mg/100 g dw), AFSR (5.0 ± 0.5 mg/100 g dw) and AF (3.1 ± 0.2 mg/100 g dw), which showed the lowest vitamin E contents, probably justified by the lower overall proportion of fat in the formulation (Annex I), either due to the addition of less sugar in AFSR, or due to the use of whole powdered almonds in AT, instead of the PDAF in AF.

Table 2. Vitamin E profile (mg/100 dry weight) for the prepared “almendrados” formulations. Values for the almond flour alone are also presented to serve as reference. The results are presented as mean±SD.¹

		α-Tocopherol	α-Tocotrienol	β-Tocopherol	γ-Tocopherol	β-Tocotrienol	δ-Tocopherol	Vitamin E
Formulation	Al	1.9±0.2 d	0.85±0.04 a	0.70±0.02 a	1.8±0.2 a	0.82±0.04	1.0±0.1 a	7.2±0.3 a
	AT	4.9±0.2 a	0.19±0.02 b	0.06±0.01 c	0.13±0.01 b	nd	0.026±0.003 b	5.3±0.2 b
	AF	2.7±0.2 c	0.11±0.02 c	0.04±0.01 c	0.15±0.02 b	nd	0.028±0.002 b	3.1±0.2 c
	AFSR	4.1±0.3 b	0.22±0.03 b	0.41±0.05 b	0.12±0.02 b	nd	0.029±0.003 b	5.0±0.5 b
Homoscedasticity ² <i>p</i> -value (n = 36)		0.387	0.057	0.004	<0.001	-	<0.001	0.034
One-way ANOVA ³ <i>p</i> -value (n = 36)		<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001
PDAF		11±1	0.40±0.03	0.13±0.02	0.32±0.03	nd	0.08±0.01	12±1

¹Differences among means were evaluated using the Tukey's HSD (homoscedastic distribution) or the Tamhane's T2 (heteroscedastic distribution) multiple comparison tests.

²Homoscedasticity among formulations was tested by means of the Levene test: homoscedasticity, *p*-value>0.05; heteroscedasticity, *p*-value<0.05.

³*p*<0.05 meaning that the mean value of the evaluated parameter of at least one formulation differs from the others (in this case multiple comparison tests were performed).

4.3 FATTY ACIDS

The FA profile obtained for each formulation, as well as that corresponding to the PDAF are presented in **Table 3**. AT, AF and AFSR showed very similar profiles, contrarily to the industrial formulation, certainly due to the inclusion of lard in this recipe. For all the “almendrados” prepared in “Confeitaria Luso” the percentage of unsaturated fatty acids were above 90%, with ≈70% of MUFA and ≈20% of PUFA. Oleic acid was clearly the most abundant FA (67-69%), followed by linoleic acid (21-23%) and palmitic acids (6.8-7.1%).

In most cases, the FA of these three formulations did not present statistically significant differences. Their overall profiles were very similar to that of PDAF, which might be considered as an interesting feature from the nutritional point of view. As it was expected the FA profile of PDAF is nearly the same as that typically characterized in almond samples [101, 105].

On the other hand, AI presented a high percentage of SFA (saturated fatty acids), mostly due to the contribution of palmitic acid (C16:0) and stearic acid (C18:0), which are among the main FA of lard.

Overall, it should be highlighted that the beneficial FA profiles presented by AT were fully maintained in AF and AFSR.

4.4 SENSORY ANALYSIS

The process of developing a new food product requires taking into account the sensory aspects and consumer acceptance. The sensory quality of “almendrados” was evaluated rating appearance, taste, sweetness, crunchiness and hardness to determine the overall quality and acceptability of the product. In addition, the buying predisposition and preference order were also evaluated.

The 74 tasters had ages varying among 13 and 75 years old, with high predominance of individuals with ages between 21 and 50 years (**Figure 14**). Regarding the gender, most of the tasters were women (57, 77%).

Independently of the formulation, the majority of the tasters liked the appearance of “almendrados” (**Figure 15**), which somehow validates the choice of this particular type of cookie. Even so, the traditional formulation was more appreciated, since 61 individuals liked the appearance of AFSR, 62 of AF and 63 of AT, but only 39 liked the appearance of AI. Interestingly, 29 tasters liked very much the formulation incorporating PDAF, which is a strong indicator of the potential acceptability of the “almendrados” added with that by-product.

Table 3. Fatty acids profile (relative percentage) for the prepared “almendrados” formulations. Values for the almond flour alone are also presented to serve as reference. The results are presented as mean±SD.¹

		C14:0	C16:0	C16:1	C18:0	C18:1n9c	C18:2n6c	C18:3n3	SFA	MUFA	PUFA
Formulation	Al	1.0±0.1 d	24±1 a	1.4±0.1 a	16±1 a	42±2 c	14±1 c	0.57±0.05	41±1 a	44±2 c	15±1 c
	AT	nd	6.9±0.4 b	0.52±0.05 b	2.1±0.3 b	69±1 a	21±1 b	nd	9±1 b	70±1 a	21±1 b
	AF	nd	6.8±0.4 b	0.48±0.03 b	2.3±0.2 b	67±1 b	23±1 a	0.04±0.01	9±1 b	68±1 b	23±1 a
	AFSR	nd	7.1±0.4 b	0.50±0.05 b	2.3±0.2 b	67±1 b	23±1 a	nd	9±1 b	68±1 b	23±1 a
Homoscedasticity ²	<i>p</i> -value (n = 36)	-	0.385	0.433	0.006	0.112	0.811	<0.001	0.127	0.129	0.842
One-way ANOVA ³	<i>p</i> -value (n = 36)	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PDAF		nd	7.0±0.4	0.54±0.05	2.6±0.3	68±1	21±1	0.24±0.05	10±1	69±1	21±1

¹Differences among means were evaluated using the Tukey's HSD (homoscedastic distribution) or the Tamhane's T2 (heteroscedastic distribution) multiple comparison tests.

²Homoscedasticity among formulations was tested by means of the Levene test: homoscedasticity, *p*-value>0.05; heteroscedasticity, *p*-value<0.05.

³*p*<0.05 meaning that the mean value of the evaluated parameter of at least one formulation differs from the others (in this case multiple comparison tests were performed).

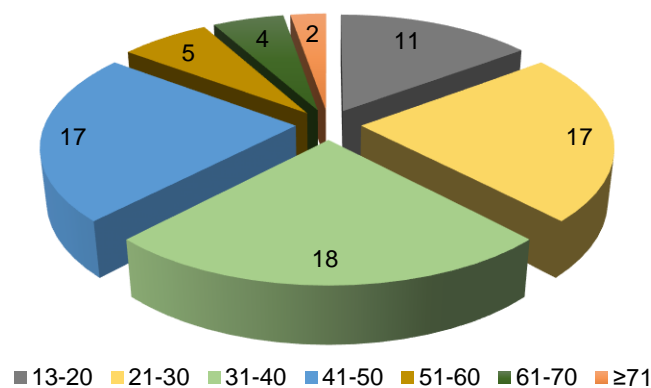


Figure 14. Age distribution of the 74 evaluating tasters.

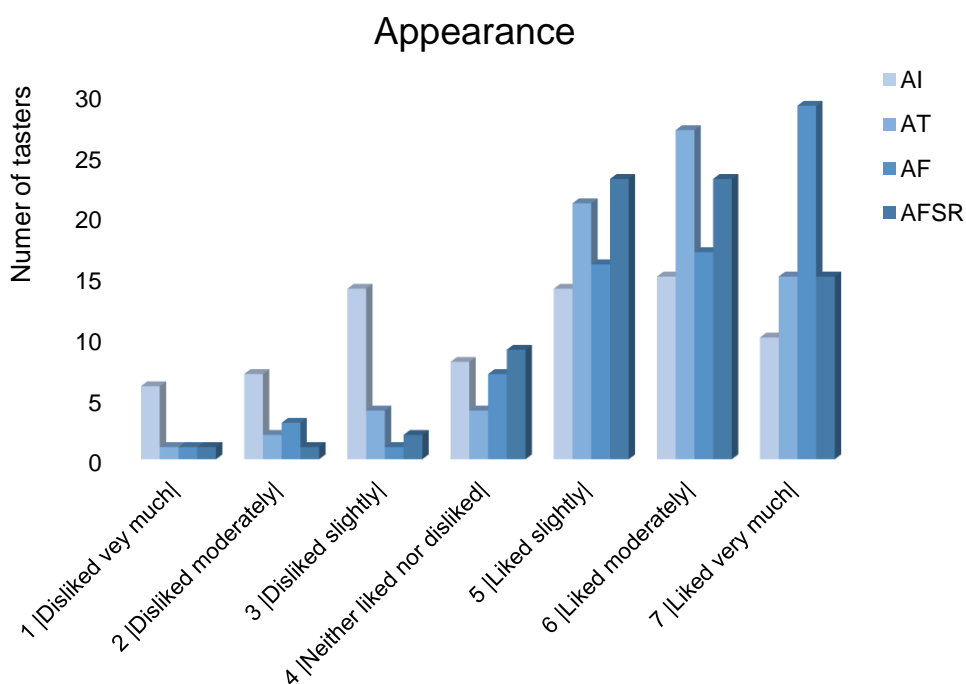


Figure 15. Evaluation scores for the appearance criterion.

The results obtained for the overall taste (**Figure 16**) are very similar to those obtained for the appearance criterion. The majority of the individuals liked the taste of “almendrados”, especially those produced at “Confeitaria Luso”. However, the number of individuals that “liked very much” was not as high as the one obtained for the appearance criterion, which might indicate a certain need towards improving the corresponding formulations.

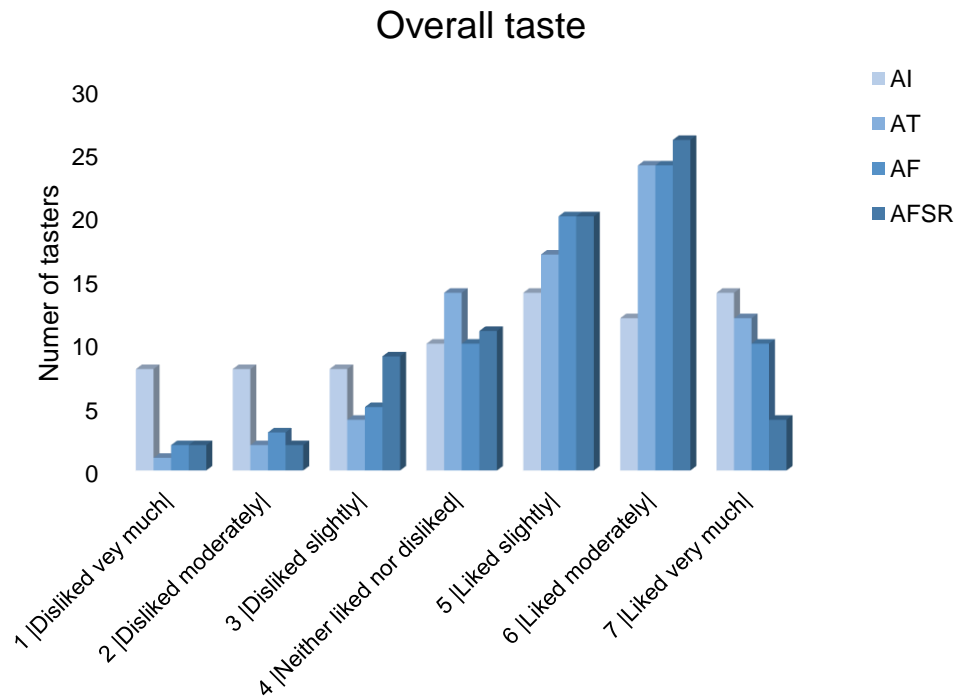


Figure 16. Evaluation scores for the overall taste criterion.

There seems to be a strong correlation among the overall taste, sweetness (**Figure 17**) and crunchiness (**Figure 18**), since the results were nearly the same for the three criteria.

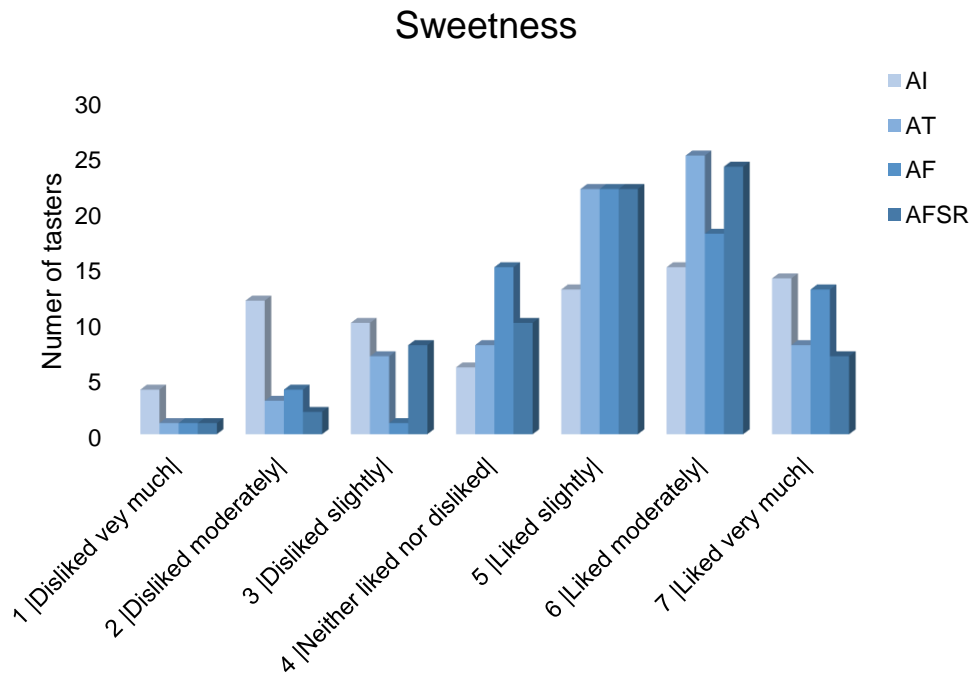


Figure 17. Evaluation scores for the sweetness criterion.

Curiously, the sugar reduction did not affect the sweetness sensation, which should be considered as an interesting result, since it indicates the possibility of reducing the sugar content in 30%, with potential health benefits, without affecting the pleasant sweet sensation. In fact, 53 individuals liked the AFSR formulation, exactly the same number of those that liked the AF formulation; 55 liked the AT formulation and only 42 indicated to like the AI “almendrados”. Among these individuals, the most frequent answer was “like moderately”, which indicates that the sugar content could be optimized, especially taking into account the low number of individuals that declared to have “liked very much”. The same conclusion could be inferred from the crunchiness results.

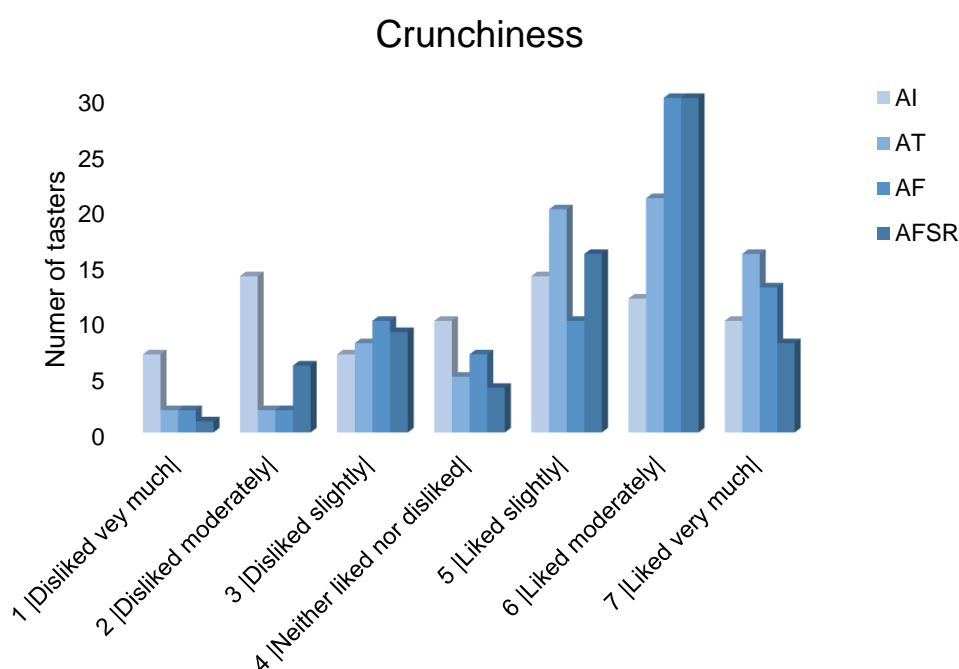


Figure 18. Evaluation scores for the crunchiness criterion.

The results obtained for “hardness” (**Figure 19**) were not as good as those acquired for the previous criteria. Despite the majority of individuals still answered to be satisfied with the hardness of “almendrados”, the number of “like slightly”, “like moderately” or “liked very much” decreased.

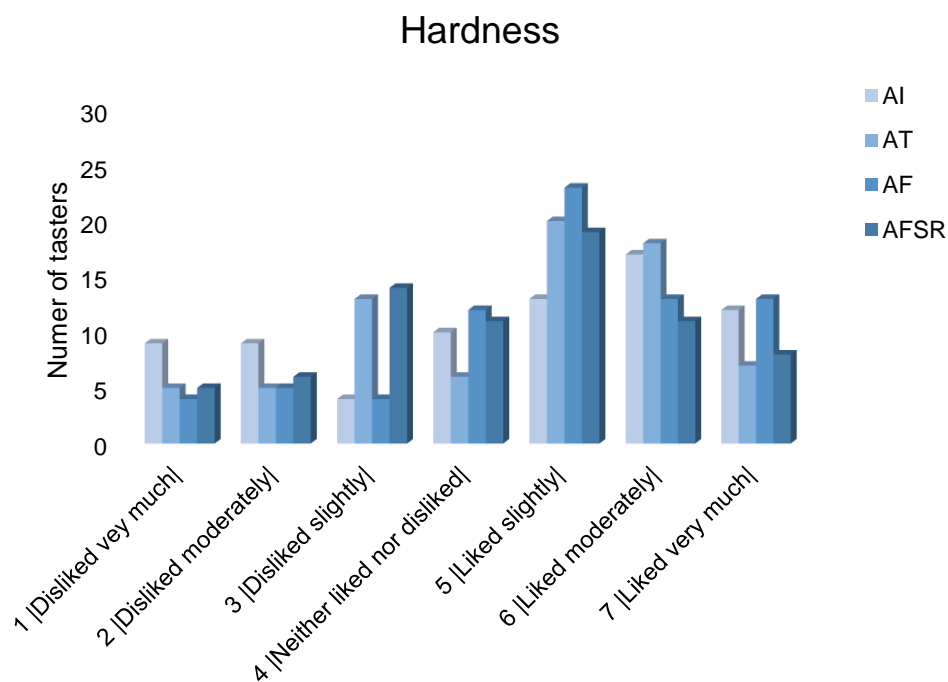


Figure 19. Evaluation scores for the hardness criterion.

Nevertheless, the results obtained for the global quality (**Figure 20**) were highly satisfactory, especially for AT (17 “liked slightly”, 18 “liked moderately”, 16 “liked very much”), AF (19 “liked slightly”, 24 “liked moderately”, 14 “liked very much”) and AFSR (20 “liked slightly”, 17 “liked moderately”, 10 “liked very much”). On the other hand, less than 50% of the tasters declared to have like of the overall quality of AI “almendrados” tasters declared to have like of the overall quality of AI “almendrados”.

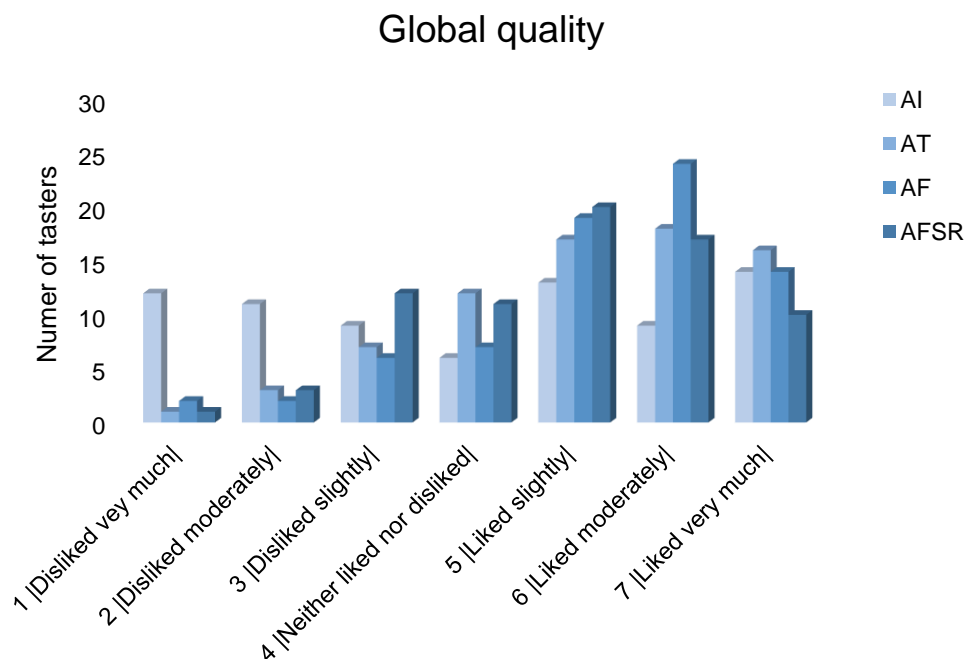


Figure 20. Evaluation scores for the global quality criterion.

The previous results are reflected in the answers obtained in the question regarding the potential buy of the tested products (**Figure 21**). In this criterion, the best scores were registered for AF (24 “I’d probably buy” and 25 “I’d certainly buy answers”), with AT (16 “I’d probably buy” and 22 “I’d certainly buy answers”) and AFSR (26 “I’d probably buy” and 10 “I’d certainly buy answers”) showing very similar results.

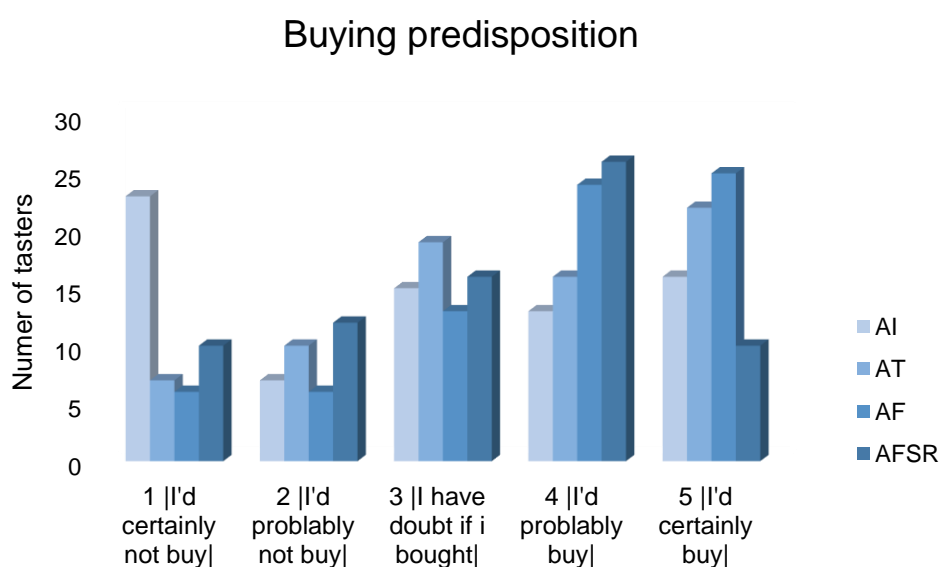


Figure 21. Evaluation scores for the “buying” predisposition criterion.

In general, the sensorial analysis highlighted AT, AF and AFSR as the best formulations. Despite the similarity in the results obtained for these three formulations, the AF formulation gathered the overall preference of the 74 tasters, as it might be observed in **Figure 22**. This result is a clear indication of the potential acceptability of “almendrados” added with the PDAF by-product.

Overall scores of all "almendrados" formulations

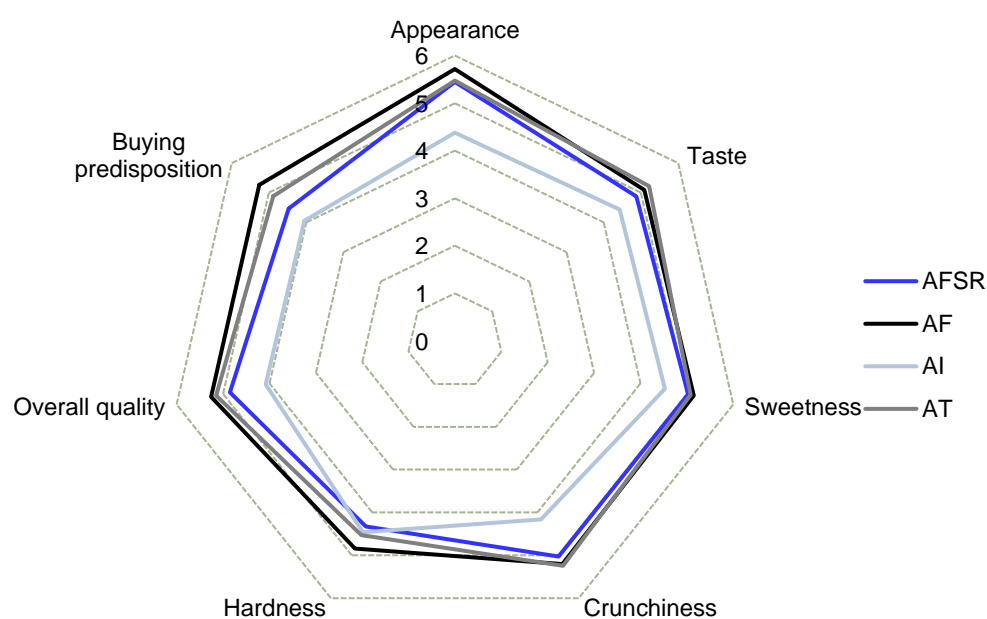


Figure 22. Overall scores for all the evaluated criteria. Results obtained for the “buying” predisposition were multiplied by a 7/5 factor.

5. CONCLUSION

The current world scenario of food industry, together with the problems associated with food security and availability, demand for integrated solutions, able to simultaneously fulfil problems related with nutritional needs, healthier food products, environmental concerns and economic constraints.

This context created the basis for the implementation of new approaches, such as it is entirely exemplified by the “circular economy” concept. Following these premises, the main purpose of this work was using a high-potential by-product (the partially delipidified almond flour) of the nut oil extraction industry to enhance the formulation of an almond-based food product. Owing their high acceptability and potential health properties, the biscuit “almendrado” was selected as the starting food. Besides characterizing samples based in the traditional formulation, industrial “almendrados” were also used.

From the nutritional point of view, as well as in what regards the vitamin E and fatty acids profiles, the studied samples indicated that the traditional-based formulations had higher quality. In general, AT, AF and AFSR, showed higher protein and minerals content, lower fat amounts, and a much higher percentage of unsaturated fatty acids (especially oleic acid and linoleic acid). In terms of total vitamin E, in the industrial formulation which showed higher levels, probably due to its higher percentage of fat.

The development of a new food product would not be significant without carrying out the sensorial analysis. Thereby, 74 tasters were randomly selected to try all the studied formulations and answer a questionnaire composed of hedonic scale based questions. Despite the overall positive results obtained with all formulations, AT, AF and AFSR showed the highest scores. Interestingly, among these three formulations, AF had the best results, clearly confirming the potential interest of partially delipidified almond flour to enhance the quality and acceptability of this highly appreciated biscuit.

Besides the advantageous effects on the consumer’s health, these results might also implement a new economic asset for the nut oil extraction industry, taking advantage of this high potential by-product, which otherwise would be included in low-value applications or simply discarded. Furthermore, the main outcomes have created the necessary basis to develop related food products, as well as to consider the use of other nuts partially delipidified flours, such as pistachio, walnut or hazelnut, which are also processed by the Spanish company that collaborated in this work.

Overall, the developed product represents an enhanced formulation, with benefits from the nutritional, environmental and economic perspectives.

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ANNEX I

1) Ingredients of “almendrados” bought at a local grocery (AI)

Wheat flour, lard, sugar, egg, almonds (4%) and leavening agents: sodium diphosphate and sodium carbonate. May contain traces of milk, soya, sesame and products thereof.

Nutritional Information of “Almendrados” bought at a local grocery (AI)

Component	100 gr
Energy	525 Kcal
Fat	29 g
Carbohydrates	58 g
Fiber	2.6 g
Protein	6.7 g
Salt	0.3 g

2) Ingredients of “Almendrados” produced at “Confeitaria Luso” (AT)

Ingredient	Quantity
White sugar	1.5 kg
Almond	0.5 kg
Potato starch	0.250 kg
Icing sugar	0.250 kg
Bread crumbs	0.150 kg
Egg whites	0.5 l
Almond flavouring	1 drop
wafer	1 unit

3) Ingredients of “Almendrados” produced at “Confeitaria Luso” added with delipidified almond flour (AF)

Ingredient	Quantity
White sugar	1.5 kg
Delipidified almond flour	0.5 kg
Potato starch	0.250 kg
Icing sugar	0.250 kg
Bread crumbs	0.150 kg
Egg whites	0.5 l
Almond flavouring	1 drop
wafer	1 unit

Ingredients of “produced at “Confeitaria Luso” added with partially delipidified almond flour and with a 30% sugar reduction (AFSR)

Ingredient	Quantity
White sugar	1.05 kg ^a
Delipidified almond flour	0.5 kg
Potato starch	0.250 kg
Icing sugar	0.250 kg
Bread crumbs	0.150 kg
Egg whites	0.5 l
Almond flavouring	1 drop
wafer	1 unit

^aCorresponding to a 30% reduction ($1.5 - 0.3 \cdot 1.5 = 1.05$).